APPLICATION NOTE

ICP – Mass Spectrometry

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Gold Nanoparticle Uptake by Tomato Plants Characterized by Single Particle ICP-MS

Introduction

With the increasing use of engineered nanoparticles (ENPs) in a variety of products and processes, there is concern about the release of ENPs into and impact on the environment. One aspect of the environmental impact of ENPs that must be explored is their uptake by plants, as ENPs can make their way to plants via migration through water and/or soil. If ENPs end up in food crops, this is a potential pathway to human exposure.

The challenge arises in how to measure ENPs in plant materials and, more specifically, in sample preparation. To our knowledge, current sample preparation techniques have limited capability to conserve the concentration and characteristics of nanoparticles (NPs) once they enter plant tissues, as they mainly depend on acid digestion. These limitations can be avoided by careful choice of the ENP extraction procedure and performing the analysis with single particle ICP-MS (SP-ICP-MS), the combination of which will preserve the particle size information, allow for rapid analysis of a large number of samples, and yield results on the particle size, concentration, and size distribution.

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The goals of this work are to develop an extraction procedure for ENPs from plant materials and perform the analysis with SP-ICP-MS. Once these steps have been established, both will be applied to the determination of gold (Au) NP uptake by tomato plants. A more detailed study of the work presented here is available[1].

Experimental

Sample Preparation

Tomato plants were grown from seeds. After developing for 29 days, the seedlings were exposed to 40 nm polyvinylpyrrolidone (PVP)-coated Au NPs (nanoComposix™, San Diego, California, USA) at different concentrations for four days before being harvested for analysis.

After harvesting, plant shoots were washed three times with deionized water, then cut into small pieces and homogenized in 8 mL of the a 2 mM citrate buffer. After homogenization, 2 mL of Macroenzyme R-10 (bioWORLD™, Dublin, Ohio, USA) was added, and the sample then shaken in a 37 °C water bath for 24 hours. The samples were then allowed to settle for one hour. A 0.1 mL aliquot of the supernatant was diluted 100x with deionized water for analysis by SP-ICP-MS. Controls and blanks were prepared the same way. Au NPs were spiked into the plant extract, as appropriate.

Instrumental Conditions

All analyses were performed on a PerkinElmer NexION® 300D/350D ICP-MS using the Nano Application Module (PerkinElmer Part No. N8140309) within Syngistix™ software. Instrumental parameters are shown in Table 1. Both particle and dissolved calibrations were performed. Au nanoparticle calibrations were performed with 30, 50, 80, and 100 nm citrate-stabilized Au nanoparticle (nanoComposix™, San Diego, California, USA). In order to see the smallest particles, the instrument's ion optics and detector were optimized for maximum sensitivity for Au 197.

Table 1. NexION 300/350D Instrumental and Analytical Parameters.

Parameter	Value
Nebulizer	Concentric (glass)
Nebulizer Flow	1.08 L/min
Spray Chamber	Baffled Cyclonic (glass)
ICP RF Power	1600 W
Analyte	Au
Mass	197 amu
Dwell Time	0.1 ms
Settling Time	0 ms
Sampling Time	100 sec
Number of Data Points Acquired	1 million per sample
Au Density	19.3 g/cm^3

Results and Discussion

Fundamental studies performed prior to analyzing plants indicated that 40 nm Au NPs could be accurately measured at concentrations as low as 1000 NPs/mL. It was important to establish the lowest concentration where Au NPs can be accurately measured, since the Au NP concentration in the plant tissues is unknown.

To assess the impact of the digestion enzyme on Au NPs, a solution of 50 nm Au NPs (2.05 x 10^5 NPs/mL) was treated with Macroenzyme R-10. Figure 1 shows the resulting particle size distribution, which indicates that the primary particle size is 50 nm. In addition, the measured particle concentration was 1.81 x 10^5 NPs/mL, an 88.3% recovery of the original concentration. These results indicate that the enzymatic digestion procedure does not affect particle-size distribution.

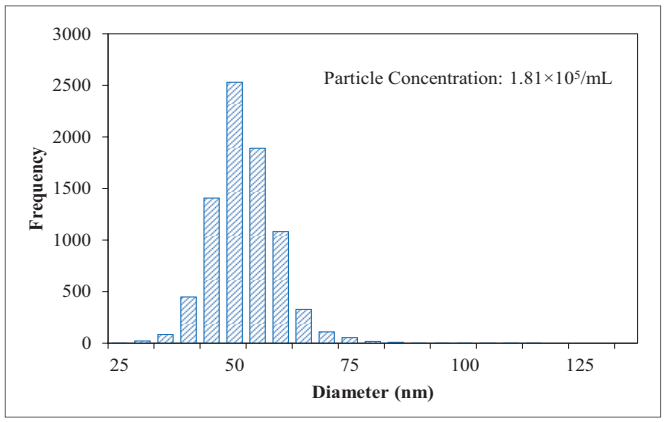

Figure 1. Particle size distribution histogram of enzyme-treated 50 nm AuNP (without plant tissue).

To establish the potential of seeing Au NPs in plants, a series of tests was performed, the results of which are shown in Figure 2. First, a solution of the Macroenzyme R-10 in a 2 mM citrate solution was analyzed for Au NPs. The resulting trace (Figure 2a) shows two random spikes, but no particles, indicating that the digestion medium will not contribute false positives.

Next, a tomato plant which had not been exposed to Au NPs was analyzed. Similar to the reagent blank, only random spikes were observed (Figure 2b). Finally, 100 nm Au NPs were added to the non-exposed digested plant (from Figure 2b) at a concentration of 4.7x10^4 NPs/mL. The resulting trace (Figure 2c) shows that the Au NPs are easily seen, with the resulting particle size distribution (Figure 2d) indicating a size distribution centered at 100 nm. These tests demonstrate that neither the enzyme nor plant material will contribute false positives, while neither inhibits the ability to detect Au NPs.

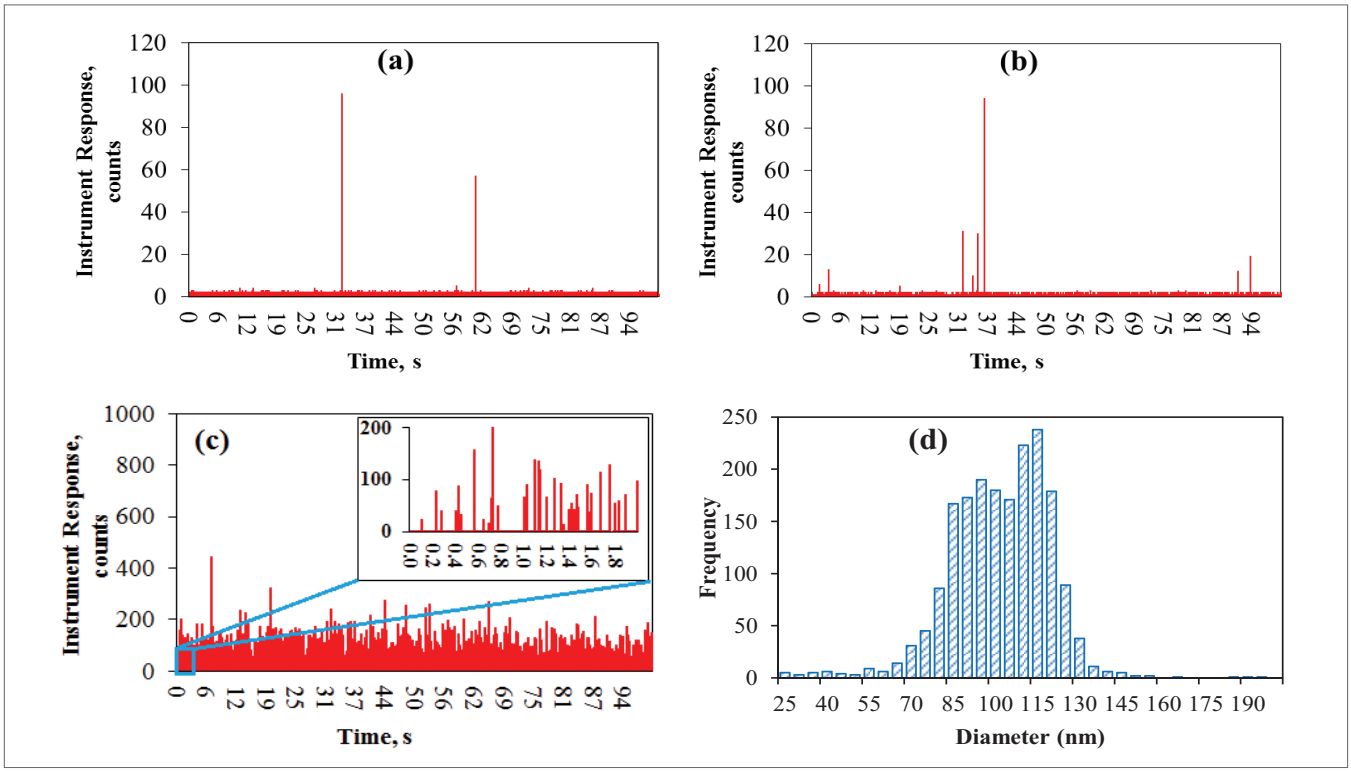

Figure 2. (a) Raw data for reagent blank (reagent blank: enzyme in 2 mM citrate solution, without plant tissues and Au NPs); (b) Raw data for control plant without exposure to Au NPs; (c) Raw data for spiking 4.7×10^4 NPs/mL of 100 nm Au NPs into control plant sample; (d) Size distribution histogram for spiking 4.7×10^4/mL of 100 nm Au NPs into control plant sample.

Next, a tomato plant exposed to 40 nm Au NPs at 5 mg/L for four days was digested and analyzed. Figures 3a and 3b show the resulting trace, which indicates uptake of the Au NPs by the plant. Figure 3c shows the resulting particle size distribution, which in centered at 40 nm, indicating the accuracy of the methodology. Finally, the same solution was spiked with 100 nm Au NPs (at $4.7\text{x}10^4$ NPs/mL) and analyzed. The resulting particle size distribution appears in Figure 3d and clearly shows two size distributions centered around 40 and 100 nm, indicating that both particle sizes are seen. In addition, the relative intensities of the distributions indicate that more 100 nm particles are present than 40 nm particles.

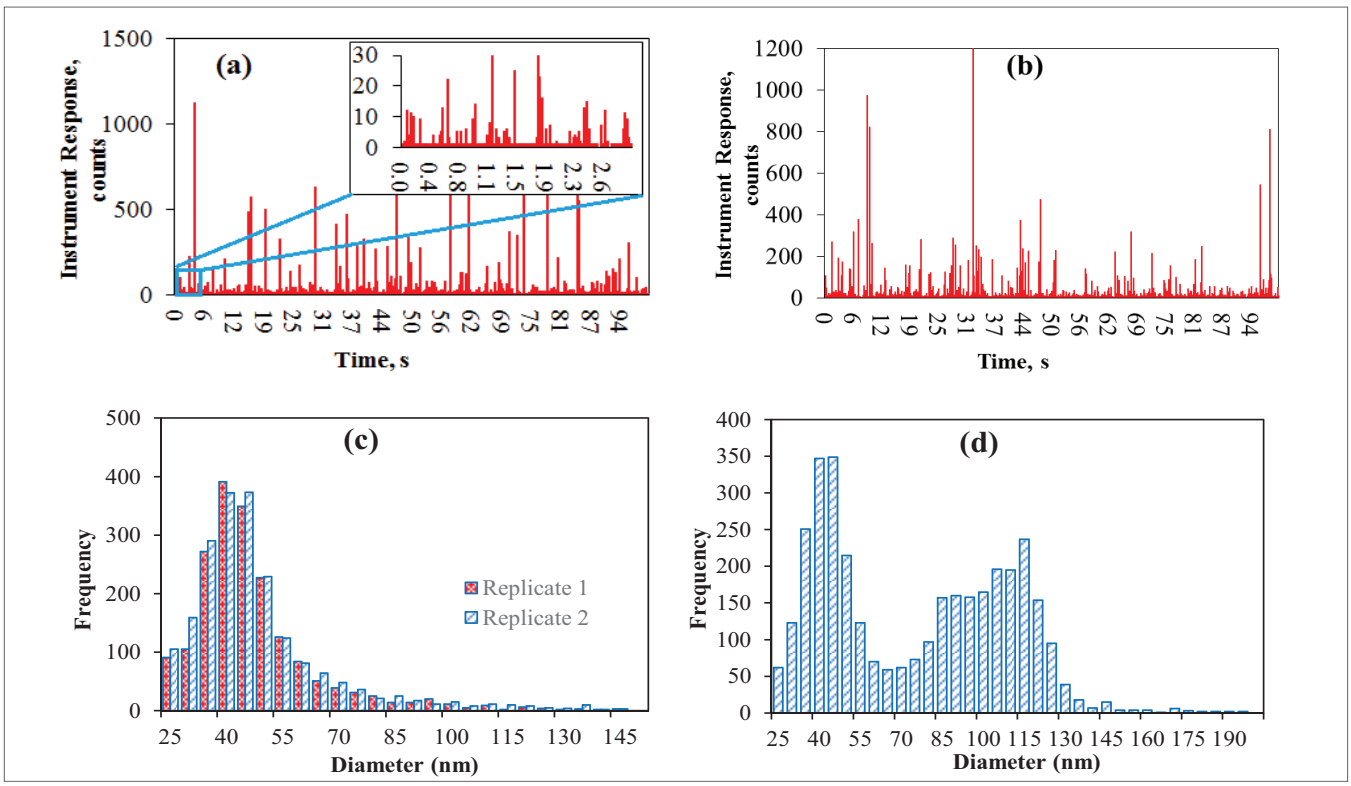

Figure 3. (a)&(b) Raw data of duplicated tomato plants exposed to 5 mg/L of 40 nm Au NPs for 4 days; (c) Size distributions histograms of duplicated tomato plants exposed to 5 mg/L of 40 nm Au NPs from Figure 4(a) and 4(b); (d) Size distribution histogram of spiking 4.7×10^4 particles/mL of 100 nm Au NPs into tomato plants exposed to 5 mg/L of 40 nm Au NPs.

Conclusions

This work has demonstrated uptake of Au NPs by tomato plants and the ability of SP-ICP-MS to detect and accurately size the particles. A digestion procedure was developed which breaks down the plant material, yet does not dissolve the Au NPs, allowing SP-ICP-MS to analyze the resulting solutions. The combination of the enzymatic digestion and SP-ICP-MS permits whole or subsections of plant shoots to be analyzed, allowing ENPs to be quickly and easily measured in plants.

References

1. Yongbo Dan, Weilan Zhang, Runmiao Xue, Xingmao Ma, Chady Stephan, Honglan Shi, 2015, "Characterization of Gold Nanoparticles Uptake by Tomato Plants Using Enzymatic Extraction Followed by Single Particle Inductively Coupled Plasma-Mass Spectrometry Analysis", *Environmental Science and Technology*, 49(5):3007-3014.

Consumables Used

Component	Part Number
Green/Orange Flared Peristaltic Pump Tubing (package of 12)	N0777042
Gold Nanoparticles in Water (30 nm)	N8142300
Gold Nanoparticles in Water (50 nm)	N8142302
Gold Nanoparticles in Water (80 nm)	N8142305
Gold Nanoparticles in Water (100 nm)	N8142307
Autosampler Tubes	B0193233 (15 mL) B0193234 (50 mL)

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